The urgent need for new anti-HIV/AIDS drugs is a global concern. In addition to obvious economical and commercial hurdles, HIV/AIDS patients are faced with multifarious difficulties associated with the currently approved anti-HIV drugs. Adverse effects, the emergence of drug resistance and the narrow spectrum of activity have limited the therapeutic usefulness of the various reverse transcriptase and protease inhibitors that are currently available on the market. This has driven many scientists to look for new anti-retrovirals with better efficacy, safety and affordability. As has always been the case in the search for cures, natural sources offer great promise. Several natural products, mostly of plant origin have been shown to possess promising activities that could assist in the prevention and/or amelioration of the disease. Many of these anti-HIV agents have other medicinal values as well, which afford them further prospective as novel leads for the development of new drugs that can deal with both the virus and the various disorders that characterize HIV/AIDS. The aim of this review is to report new discoveries and updates pertaining to anti-HIV natural products. In the review anti-HIV agents have been classified according to their chemical classes rather than their target in the HIV replicative cycle, which is the most frequently encountered approach. Perusal of the literature revealed that most of these promising naturally derived anti-HIV compounds are flavonoids, coumarins, terpenoids, alkaloids, polyphenols, polysaccharides or proteins. It is our strong conviction that the results and experiences with many of the anti-HIV natural products will inspire and motivate even more researchers to look for new leads from plants and other natural sources. Copyright © 2005 John Wiley & Sons, Ltd.
**FLAVONOIDS**

Flavonoids and related polyphenols possess promising anti-HIV activity. A number of flavonoids inhibit reverse transcriptase (RT), induce interferons and inactivate viral protease (Havsteen, 2002), and down-regulate the expression of HIV co-receptors such as CCR2b, CCR3 and CCR5 (Nair et al., 2002). However, the molecular mechanisms underlying the anti-HIV effects of flavonoids and polyphenolic compounds still need to be clearly elucidated (Nair et al., 2002).

Flavonoid constituents of a proprietary grape seed extract which are predominantly flavans and proanthocyanidins significantly downregulated the expression of the HIV-1 entry co-receptors, CCR2b, CCR3 and CCR5 in normal peripheral blood mononuclear cells (PBMC) in a dose dependent manner (93% viability of PBMC at 5 mg/mL). Analysis of the mechanisms underlying the anti-HIV-1 effects of grape seed extracts may help to identify promising natural products useful in the prevention and/or amelioration of HIV-1 infection. Grape proanthocyanidins have a potential value as an adjunct nutritional supplement, along with the existing conventional therapeutic regimens, in the treatment of HIV infection (Nair et al., 2002).

Baicalin (1) is an anti-HIV flavonoid obtained from Scutellaria baicalensis which is one of the seven medicinal plants constituting Sho-saiko-to, a traditional Chinese as well as a Japanese medicinal drug (Ohtake et al., 2004). Baicalin inhibits HIV-1 replication in PBMC in a dose dependent manner with IC_{50} values of 0.2–0.5 µg/mL and a suitable safety profile; well tolerated up to 10 µg/mL (Kitamura et al., 1998; Li et al., 1993). Baicalin also inhibited HIV-1 RT with an IC_{50} value of 2 µg/mL and IC_{100} ~10 µg/mL, without
Figure 1. (Continued)
Figure 1. (Continued)
affecting the DNA polymerases α and γ but slightly inhibiting DNA polymerase β (Kitamura et al., 1998). Baicalin can discriminate between double-stranded DNA and single-stranded DNA since it binds to DNA through intercalation (Sun et al., 2004). This monoglycosylated flavonoid inhibited HIV-1 Env protein mediated fusion with both CD4/CXCR4 and CD4/CCR5 cells (IC50 = 4 µM). However, since it did not inhibit binding of HIV-1 gp120 to CD4 it may interact with HIV-1 Env domains repressing their interaction with chemokine co-receptors and block HIV-1 entry of target cells (Li et al., 2000). Reports also indicate that baicalin selectively induces apoptosis in HIV-infected cells (Wu et al., 1995) and human cancer cells (Ikekze et al., 2001; Ueda et al., 2002). Thus baicalin and its analogues are potentially very useful for developing novel anti-HIV-1 agents.

Two flavonoids, 4,6-dihydroxy-2-methoxy-3-methyl-5′(3′-hydroxy)-cinnamoylbenzaldehyde (a chalcone) and lawinal (a flavanone) isolated from Thalassia testudinum (Juglandaceae) showed a very potent inhibitory activity of both LAI/IIIB and Bal strains of HIV-1 RT (IC50 < 100 µM; quercetin derivatives being more active than those of kaempferol). Moreover, the compounds bearing a feruloyl moiety exhibited higher activity than those bearing a sinapoyl moiety. Quercetin 3-O-[[(6-O-feruloyl)-β-D-glucopyranosyl-(1→2)]-β-D-galactopyranoside] and quercetin 3-O-[(6-O-sinapoyl)-β-D-glucopyranosyl-(1→2)]-β-D-galactopyranoside also inhibited HIV-1 integrase with IC50 values of 5 and 7 µM, respectively. With regard to integrase inhibitory activity, compounds possessing a feruloyl or sinapoyl group in the terminal glucose moiety showed more potent inhibitory activity than the unsubstituted ones. The aforementioned flavonoids showed higher inhibitory activity than their aglycones, quercetin (IC50 = 43 and 15 µM, respectively) and kaempferol (IC50 > 100 and 40 µM, respectively) (Tewtrakul et al., 2002). The tetrahydroxylflavonol kaempferol (6) (Rosa damascena) effectively reduced the maturation of infectious progeny virus apparently due to selective inhibition of the viral protease (IC50 = 0.8 µM, TI = 62.5 in H9 cells). On the other hand, the pentahydroxylflavonol quercetin (7) prevented binding of gp120 to CD4 (IC50 = 10 µM, TI = 10 in H9 cells) (Mahmood et al., 1996). Furthermore, two related flavonol glucosides namely, (−)-4′-methylepigallocatechin-3′-O-β-glucopyranoside and (−)-4′′-methylepigallocatechin-3′-O-β-glucopyranoside isolated from the Sudanese medicinal plant Maytenus senegalensis exhibited 72.9% and 68.2% inhibition of HIV-1 protease, respectively, at a concentration of 100 µM (Hussein et al., 1999).

(−)-Epigallocatechin-3-gallate (EGCG) which is approximatively 50% of the total catechin content of green tea represents a potential low-cost inhibitor of HIV infection that could be associated with current anti-HIV therapy. Related compounds like (−)-epicatechin and (−)-epicatechin-3-O-gallate from Detarium microcarpum were reported to block HIV infection through an irreversible interaction with the glycoprotein gp120: IC50 2 and 1 µg/mL, respectively, in C8166 cells and a CC50 value of >100 µg/mL (Mahmood et al., 1993); Nakajima and Ono (1990) reported that (−)-epicatechin gallate and EGCG, the two components from the tea plant Camellia sinensis, differentially inhibit the activities of RT with IC50 values in the range of 0.01–0.02 µg/mL, whilst Fassina et al. (2002) recently reported EGCG to inhibit HIV-1 RT (both of LAI/IIIB and Bal) with doses of 25 and 50 µM giving nearly 100% inhibition. Epicatechin gallate and EGCG also inhibited other cellular DNA and RNA polymerases, in cell-free...
chemical assays. The mode of inhibition of RT and other DNA polymerases was competitive with respect to the template-primer, whereas in the case of RNA polymerase it was with respect to the nucleotide substrate (Nakane and Ono, 1990).

The mechanism by which EGCG interferes with viral infection is not yet clear, however, there are indications that it impinges on various steps of the HIV life cycle, in addition to its inhibitory effect on RT. EGCG destroyed virions by binding to the surface of the viral envelope and deforming of the phospholipids in a manner similar to the effect of polymyxin B on bacterial membranes. It does not have an appreciable effect on virus-cell binding (only 20% at 100 µM). EGCG significantly inhibited in a dose-dependent manner, entry of the virus after adsorption in monocytoid cells (THP-1) and monocyte-derived macrophages (MDM) but not in H9 cells. It also inhibited viral production in chronically infected monocytoid cells (H11B/THP-1) with an IC₅₀ value of ~20 µM but not in T-lymphoid cells (H11B/H9, MN/H9). The ability of EGCG to decrease viral mRNA production in lipopolysaccharide (LPS)-activated chronically HIV-1-infected cells (H11B/THP-1), but not in unstimulated or LPS-stimulated T-lymphoid cells (H9) suggests that its action could be related to the NF-κB pathway, which is activated by LPS-stimulation, and that EGCG may not have a strong and direct downregulatory effect on the HIV-1 promoter, which controls viral gene regulation. Similarly, the anti-protease activity of EGCG was observed only with monocytoid host cells at a concentration higher than 10 µM. Aside from obvious differences in their cell surface receptors, their responses to several stimulators, multi-drug resistance (MDR) pumps, and behaviour during viral infection between monocytes and T-cells, phagocytosis of the EGCG by monocytoid cells might also account for the above observation. Anti-HIV viral activity of EGCG may thus result from an interaction with several steps in the HIV-1 life cycle. Moreover, EGCG was not significantly cytotoxic; LD₅₀ = 174.8 µM for H9; 440.3 µM for THP-1 (Fassina et al., 2002; Yamaguchi et al., 2002).

A new class of HIV-1 RT inhibitors (8–12) obtained by the systematic structural simplification of epicatechin and epigallocatechin gallates is now recognized. Some of these compounds (8–10) inhibited the native as well as the A17 double mutant (K103N Y181C) forms of the enzyme. Substantial separation of polymerase and DNA-strand-transfer inhibition was achieved with compounds 11 and 12 for both wild-type and mutant enzyme. The last two compounds inhibit DNA-strand-transfer with up to 80-fold selectivity over polymerase activity. The presence of polar hydroxyl groups on the aromatic ring of the chromanol moiety enhanced polymerase inhibition while complete removal of these hydroxyl groups or their conversion to less polar methyl ether functions resulted in 10- to 80-fold selectivity for DNA-strand-transfer inhibition over polymerase inhibition. Removal of one or more of the hydroxyl groups on the gallic acid moiety led to a loss of both inhibitory activities. DNA-strand-transfer is rate-limiting in the overall process of reverse transcription and critical to recombination-associated mutation of the virus. Such specific DNA-strand-transfer inhibitors may thus have important therapeutic potential (Tillekeratne et al., 2002).

The flavonoid chrysin (13) and benzothiophenes have been shown to prevent HIV expression in latently and chronically infected cells (Critchfield et al., 1996) through the inhibition of casein kinase II, a cellular protein that may regulate HIV-1 transcription by phosphorylating cellular proteins involved in the HIV-1 transcription transactivation process (Critchfield et al., 1997). The mechanism of action of these compounds is independent of the nuclear factor κB-driven transcription pathway and they have demonstrated specificity toward inhibiting HIV-1 transcription (Butera et al., 1995). This unorthodox approach is in contrast with the popular view that dormancy of HIV infected cells is a major obstacle to controlling or curing HIV-1 infection (Blankson et al., 2002; Chun and Faucci, 1999). According to the latter view, breaking latency in HIV-infected cells could reduce the number of latently infected cells by causing them to be directly killed by the cytopathic action of the virus, to be recognized and destroyed by the immune system, or to express proteins that render them susceptible to targeted therapeutics such as immunotoxins (Bocklandt et al., 2003). Chrysin has low oral bioavailability in humans (estimated to be 0.003%–0.02%), mainly due to extensive presystemic intestinal as well as hepatic metabolism and efflux of metabolites (chrysin glucuronide and chrysin sulphate) back into the intestine for hydrolysis and faecal elimination. Other flavonoids as well could possibly have a similar bioavailability profile (Walle et al., 1999; Walle et al., 2001). The usefulness of chrysin and similar HIV transcription inhibitors or latency inducers remains to be assessed.

A flavonoid glucuronide, apigenin 7-O-β-D-(4’-caffeoyl)glucuronide isolated from the flowers of Chrysanthemum morifolium, showed strong HIV-1 integrase inhibitory activity (IC₅₀ = 7.2 ± 3.4 µg/mL) and anti-HIV activity in a cell culture assay (EC₅₀ = 41.86 ± 1.43 µg/mL) using HIV-1 unstable MT-4 cells (Lee et al., 2003). Similarly flemmyrulin, quercetin, eucheartin M and formosanatin C isolated from the methanol extract of Euchresta formosana inhibited HIV replication in H9 lymphocyte cells (Lo et al., 2003). Moreover, the flavonoids 6,8-diprenylaromadendrin, 6,8-diprenylkaempferol and lonchocarpol A obtained from Monotes afric anus were reported to exhibit HIV-inhibitory activity in the XTT-based, whole-cell screen. It is worth noting that all the three flavonoids contain a 5,7-dihydroxy-6,8-diprenyl system in their A ring (Meragelman et al., 2001).

The methanol and ethyl acetate extracts from a new chemotype of Mentha longifolia that grows in the Moroccan mountains significantly inhibited (p < 0.01) HIV-1BaL infection by approximately 40% and 55%, respectively. In addition, the ethyl acetate extract showed significant (p < 0.008) inhibitory activity (50% inhibition) against HIV-1 RT. Chemical analysis of these extracts suggests that flavonoids, mainly flavones may be the major inhibitors of HIV infection (Amzazi et al., 2003).

**COUMARINS**

(+)-Calanolide A (14) and related coumarins isolated from various Calophyllum spp. represent a novel and
Several triterpenoids have been found to exhibit antiretroviral activity with different mechanisms of action. The limonoids, limonin (19) and nomilin (20), inhibited HIV-1 replication in PBMC including those with chronic infection and on monocytes/macrophages with EC50 values ranging from 20 to 80 μM. HIV-1 protease seems to be their target (Battinelli et al., 2003). Another limonoid, clausenolid-1-ethyl ether isolated from the rhizomes and the roots of Clausena excavata showed anti-HIV activity in a syncytium assay with an EC50 value of 34.4 μM exhibiting a substantially low cytotoxicity (IC50 = 548 μM). The compound was confirmed to be inactive against HIV-1 RT (Sunthitikawinsakul et al., 2003). On the contrary, cycloartenol ferulate (IC50 = 2.2 μM), 24-methyleneoctanol ferulate (IC50 = 1.9 μM), lupenone (IC50 = 2.1 μM), betulin diacetate (IC50 = 1.4 μM) and karoundiol 29-benzoate (IC50 = 2.2 μM) inhibited purified HIV-1 RT and have been suggested as potential lead compounds (Akihisa et al., 2001).

3-β-Hydroxy-lup-20(29)-en-28-oic acid (betulinic acid) (21) is a pentacyclic lupane-type triterpene that is widely distributed throughout the plant kingdom. Among its many biological activities it is highly regarded for its anti-HIV-1 activity and specific cytotoxicity against a variety of tumour cell lines. Interest in developing even more potent anti-HIV agents based on betulinic acid has led to the discovery of a host of highly active derivatives exhibiting greater potencies and better therapeutic indices than some current clinical anti-HIV agents. While its mechanism of action has not been fully determined, it has been shown that some betulinic acid analogues disrupt viral fusion to the cell in a post-binding step through interaction with the viral glycoprotein gp41, whereas others disrupt assembly and budding of the HIV-1 virus, inhibition of the P24/p2 cleavage site being responsible for the antimaturation activity of the latter group, and a third group capable of inhibiting both steps of the virus replicative cycle has recently been reported. The targets of betulinic acid derivatives are varied, depending primarily on the side chain structures of the compounds (Cichewicz and Kouzi, 2004; Huang et al., 2004).

The betulinic acid derivative RPR103611 (22) blocks HIV infection at an IC50 of approximately 10 nm, through inhibition of a post-binding, envelope-dependent step involved in the fusion of the virus with the cell membrane (Mayaux et al., 1994). The target for the anti-HIV action of RPR103611 is the HIV-1 glycoprotein gp41. HIV resistance to RPR103611 is associated with amino acid substitutions at positions 22 (Arg → Ala) and 84 (Ile → Ser) of gp41 (Labrosse et al., 1997). RPR103611 is active against CCR5-dependent (X4) HIV-1 strains, such as HIV-1_LAI (LAI). Other X4 strains, such as HIV-1HOK (NDK), and CCR5-dependent (R5) HIV-1 strains, such as HIV-1ADA (ADA), were totally resistant to RPR103611. A single difference at position 91, leucine in LAI and histidine in NDK, apparently accounted for their sensitivity or resistance to RPR103611 indicating that nonpolar residues in this region are important for the antiviral activity of RPR103611 and are possibly part of its target. However, another mechanism had to be envisaged to explain the drug resistance of ADA, since its gp41 loop region was almost identical to that of LAI. Fusion mediated by chimeric Env consisting of LAI gp120 and ADA gp41, or the reciprocal construct, was fully blocked by RPR103611. The gp120-gp41 complex of R5 strains is stable, relative to that of X4 strains, and this stability could play a role in their drug resistance. Therefore, the antiviral efficacy of RPR103611 depends on the sequence of the gp41 loop and the stability of the gp120-gp41 complex, which could limit the accessibility.
of this target (Labrosse et al., 2000). On the other hand, gp120 appears to be the target of the stereo-isomer of RPR103611, IC9564 (Holz-Smith et al., 2001).

Hydrogenation of betulinic acid yielded dihydrobetulinic acid, which showed an IC₅₀ of 0.9 μM and a selectivity index of 14. 3-O-(3′,3′-Dimethylsulfinyl)betulinic acid (PA-457) and 3-O-(3′,3′-dimethylsulfinyl) dihydrobetulinic acid have remarkably high anti-HIV activity and selectivity; IC₅₀<0.35 nm: selectivity index >20 000 and >14 000, respectively (Kashiwada et al., 1996). PA-457 inhibited replication of patient-derived WT viruses; IC₅₀ of respectively (Kashiwada et al., 1996). PA-457 inhibited replication of patient-derived WT viruses; IC₅₀ of 10.3 nm, TI >2500. It was also active against a panel of virus isolates resistant to various anti-reverse transcriptases and antiproteases with a mean IC₅₀ value of 7.8 nm. PA-457 acts by disrupting a late step in Gag processing involving conversion of the capsid precursor (p25) to mature capsid protein (p24). PA-457 was inactive against the related retroviruses HIV-2 and simian immunodeficiency virus (SIV) in cell-based replication assays. PA-457 represents a unique class of anti-HIV compounds referred to as maturation inhibitors. Such compounds act on a previously unexploited viral target, providing additional opportunities for HIV drug discovery (Li et al., 2003). Other betulinic acid derivatives such as LH15 and LH55 exhibit the aforementioned activities combined, i.e. they are antienty like IC9564 and antimaturation like PA-457 (Huang et al., 2004).

Glycyrrhizin (GL) from licorice root (Glycyrrhiza glabra) has been known for some time as an antiviral agent, its IC₅₀ against HIV-1 in MT-4 cells being 0.15 μM. Its effect was thought to be mediated at least partly through inhibition of protein kinase C (PKC) and interference with virus–cell binding, although the site of interaction of glycyrrhizin at the envelope glycoprotein has not been further characterized (Ito et al., 1988). Hattori et al. (1989) have demonstrated its in vivo effects in AIDS patients. Glycyrrhizin has the potential to inhibit a non-syncytium-inducing variant of HIV (NSI-HIV) replication in PBMC cultures by inducing the production of β-chemokines (Sasaki et al., 2002–2003). In addition to this, it suppressed in vitro UV-induced HIV gene expression in stably transfected HeLa HIV-LTRcat cells, without affecting cell proliferation and viability at doses as high as 2.4 nm. The inhibitory effect correlated with the complete inhibition of binding activities of NF-xB p65, NF-xB p50, c-Fos and c-Rel (Cheng et al., 2004). Some of the chemically modified glycyrrhizin derivatives (salts, amides, glycopeptides) were potent HIV-1 and HIV-2 inhibitors in vitro. An example of these is niglizin (penta-O-nicotinate of GL) (Baltina, 2003). Persons with HIV may have previous or concurrent liver impairment as a result of injection drug use, hepatitis, alcohol abuse and damage from medication. Additional stress is placed on the liver by low-grade opportunistic infections and haemoptysis. It is especially important that people with HIV care for their liver to help this organ remain physiologically normal during chronic and acute management of HIV infection. Readily available liver protectants such as thiotic acid, glycyrrhizin and Silibum marianum are very important in this aspect (Hernandez, 1995).

Oleanolic acid was identified as an anti-HIV principle from several plants, including Rosa woodsii (leaves), Proscipis glandulosa (leaves and twigs), Phoradendron juniperinum (whole plant), Syzygium claviflorum (leaves), Hypis capitata (whole plant) and Ternstroemia gymnanthera (aerial part). It inhibited HIV-1 replication in acutely infected H9 cells with an EC₅₀ value of 1.7 μg/mL and a TI of 12.8. Pomolic acid, isolated from R. woodsii and H. capitata, was also identified as an anti-HIV agent (EC₅₀ 1.4 μg/mL, TI 16.6). Although ursolic acid did show anti-HIV activity (EC₅₀ 2.0 μg/mL), it was shown to be slightly toxic (IC₅₀ 6.5 μg/mL). A derivative of oleanolic acid, on the other hand, demonstrated a very potent anti-HIV activity, with an EC₅₀ value of 0.0005 μg/mL and a TI value of 22 400 (Kashiwada et al., 1998).

It has been reported that the use of a sterols/storin mixture in HIV infection shifts the balance of type 1 T helper cells to type 2 helper cells (Th1/Th2) towards the more beneficial Th1 and also maintains CD4 cell numbers over an extended period of time in the absence of any anti-retroviral therapy (Breytenbach et al., 2001).

A phase I dose-escalating clinical trial of andrographolide, a diterpenoid lactone (Andrographis paniculata) previously reported to inhibit cell to cell transmission, viral replication and syncytia formation in HIV infected cells, revealed a significant rise in the mean CD4¹ lymphocyte level from a baseline of 405 cells/mm³ to 501 cells/mm³; p = 0.002) of HIV-1 infected subjects after 2 weeks administration of 10 mg/kg andrographolide. No subjects used antiretroviral medications during the trial. Unlike in the sero-positive subjects there was an unexplained decrease in CD4¹ counts in all sero-negative counterparts, indicating a possible differential effect. The plasma viral load did not significantly decrease throughout the trial. It is proposed that andrographolide may inhibit HIV-induced cell cycle dysregulation, rather than interrupting viral replication directly. The side effects experienced by the cohorts and rated as being mild to moderate include headache, fatigue, rash, bitter/metallic/decreased taste, loose stool/diarrhoea and pruritus. Anaphylactic reactions have also been reported in one HIV-positive subject (Calabrese et al., 2000).

Agents that induce HIV-1 out of latency would be useful adjuvants for currently available anti-retroviral therapy. 12-Deoxyphorbol-13-phenylacetate (DPP), an anti-tumour-promoting phorbol ester originally isolated from the West African plant Euphorbia poissonii, induced the expression of HIV-1 in latently infected T cells (IC₅₀ = 4 nm, TI = 11 500) and rendered them sensitive to killing by an immunotoxin targeted to the viral envelope glycoprotein. DPP also regulates an extensive series of genes under the control of PKC, including several involved in T cell activation and cytoskeleton reorganization, and represses expression of the HIV-1 receptor CD4 (IC₅₀ = 14 nm) and coreceptor CXCR4 (IC₅₀ = 2.9 nm). DPP is 20- to 40-fold more potent than the related phorbol ester prostratin, probably due to its more lipophilic aromatic side chain structure at position 13. The combination of high potency and anti-tumour promoting activity make DPP an attractive candidate for the adjunctive therapy of persistent HIV-1 infection (Bocklandt et al., 2003). Another phorbol ester, pedilustin [13-O-acetyl-12-O-(2',3',4',5'-octadienoyl)-4α-deoxyphorbol], an anticancer principle from Pedilanthes sp., was shown to afford protection.
(to 80%) of human-derived lymphoblastoid CEM-SS cells from infection and cell-killing by HIV-1, at concentrations of 2–5 μM and also inhibited PKC with a K<sub>i</sub> of 620 ± 20 nM (Pettit et al., 2002). Similarly, 12-O-tetradecanoylphorbol-13-acetate from the seeds of <i>Croton tiglium</i> was found to be a potent inhibitor of HIV-1-induced cytotoxic effect on MT-4 cells with complete inhibitory concentration (IC<sub>100</sub>) value of 0.48 ng/mL and minimum cytotoxic concentration (CC<sub>50</sub>) value of 31.3 μg/mL. In addition, it was found to be an effective activator of PKC (96% activation at 10 ng/mL). Also, 12-O-acetylphorbol-13-decanoate effectively inhibited the cytotoxic effect of HIV-1 on MT-4 cells with IC<sub>100</sub> value of 7.6 ng/mL and CC<sub>50</sub> value of 62.5 μg/mL (El-Mekkawy et al., 2000). Table 1 summarizes other terpenoid-derived anti-HIV compounds.

### ALKALOIDS

Screening of natural products in the search for human CCR5 inhibitors led to the identification of anibamine, a novel pyridine quaternary alkaloid as a trifluoroacetic acid salt, from <i>Aniba</i> sp. Anibamine-TFA competed for the binding of 125I-gp120 to human CCR5 with an IC<sub>50</sub> of 1 μM. Formation of the TFA salt of anibamine is plausibly an artifact of the isolation process (Jayasuriya et al., 2004). Similarly, the pentacyclic guanidine alkaloids crambescidin 826, crambescidin 800 and fromiamycinol isolated from the marine sponge <i>Monanchora</i> sp. inhibit HIV-1 envelope-mediated fusion <i>in vitro</i> with IC<sub>50</sub> values of 1–3 μM (Chang et al., 2003). Other marine alkaloids named manadomanzamines A (26) and B (27) which are structurally related to the manzamine-type alkaloids, have been isolated from an Indonesian sponge <i>Acanthostrongylophora</i> sp. (Haplosporida: Penthosidae) and characterized to have significant activities against <i>Mycobacterium tuberculosis</i> and HIV-1, and moderate activity against several AIDS opportunistic fungal infections. Manadomanzamines A and B are active against HIV-1 with EC<sub>50</sub> values of 7.0 and 16.5 μg/mL, respectively. Manadomanzamine A is also active against human lung carcinoma A-549 and human colon carcinoma H-116, while manadomanzamine B is only active against the latter. In addition, xestomanzamine A another alkaloid from the same sponge species was active against HIV-1 at IC<sub>50</sub> of 11.2 μg/mL. Manadomanzamines A, B and xestomanzamine A did not show cytotoxicity against the normal Vero cell line (African Green Monkey kidney cells) at a concentration of 4.8 μg/mL. Manadomanzamine B and xestomanzamine A are also active against the fungus <i>Cryptococcus neoformans</i> with IC<sub>50</sub> values of 3.5 and 6.0 μg/mL, respectively. Manadomanzamine A was active against <i>Candida albicans</i> with an IC<sub>50</sub> of 20 μg/mL (Peng et al., 2003).

One of the natural products with interesting activity on RT is polycitone A (28), an aromatic alkaloid isolated from the marine ascidian <i>Polyctorus</i> sp. Polycitone A exhibits potent inhibitory capacity of both RNA- and DNA-directed DNA polymerases, such as reverse transcriptases of various retrovirals, such as HIV-1 (including L74V, Q89G, Y183F and M184L mutants), HIV-2, murine leukemia virus and mouse mammary tumour virus (MMTV), <i>Escherichia coli</i> DNA polymerase I and cellular α and β DNA polymerases. The IC<sub>50</sub> values for inhibition of the RNA- and DNA-directed DNA polymerase functions of HIV-1 RT were as low as 245 nM and 470 nM, respectively. As to its mode and mechanism of inhibition of HIV-1 RT, experimental evidence suggests that the inhibition of the DNA polymerase activity is independent of the template-primer used and also does not appreciably affect the RNase H function (IC<sub>50</sub> = 30 μM). Polycitone A, on the other hand, has been shown to interfere with DNA primer extension (IC<sub>50</sub> = 2.5 μM) as well as with the formation of the RT-DNA complex (IC<sub>S0</sub> = 5–10 μM). To add further detail to this, it seems that polycitone A inhibits the HIV-1 RT polymerase by preventing its reassociation with the DNA primer after it dissociates from the template-primer during extension. Steady-state kinetic studies also demonstrated that polycitone A can be considered as an allosteric inhibitor of HIV-1 RT that decreases the affinity of the enzyme to its substrate. Furthermore, despite the fact that polycitone A bears no structural relationship to dTTP, it is a competitive inhibitor with respect to dTTP indicating that the inhibitor binding site on the enzyme may be functionally or spatially related to the substrate binding site. Natural and chemical derivatives in which some or all of the five phenol groups have been methoxylated showed substantially decreased inhibition of HIV-1 RT DNA polymerase activity, signifying the importance of the hydroxyl groups of polycitone A. In pentamethoxy polycitone A, for instance, the abilities to inhibit DNA primer extension as well as the formation of the RT-DNA complex were absent. Although polycitone A (similar to toxisul) is a general inhibitor of DNA polymerase, lacking specificity to retroviral reverse transcriptases, its inhibition of the first step in DNA polymerization, i.e. the formation of the RT–DNA complex, and hence, of the overall process, could serve as a model for the rational design of new selective anti-HIV RT derivatives and possibly anti-AIDS drugs (Loya et al., 1999).

The aporphine alkaloids heronidine (29), laurolistine (30), 7-oxohernangericine and lindechunine A isolated from the roots of <i>Lindera chunii</i> showed significant anti-HIV-1 integrase activity with IC<sub>50</sub> values of 16.3, 7.7, 18.2 and 21.1 μM, respectively (Zhang et al., 2002). The alkaloid harman isolated from <i>Symphlocos setchuenensis</i> was found to inhibit HIV replication in H9 lymphocyte cells with an EC<sub>50</sub> of 10.3 μM and TI of 7.5. It was derivatized to give a compound showing a potent activity with EC<sub>50</sub> and TI values of 0.037 μM and 210, respectively (Ishida et al., 2001). Similarly, a sesquiterpene pyridine alkaloid triptitone B (31) isolated from <i>Tripterygium hypoglaucum</i> and a clinically used extract of <i>T. wilfordii</i>, demonstrated potent inhibition of HIV-1 replication in H9 cells with an EC<sub>50</sub> < 0.10 μg/mL and TI value of >1000 (Duan et al., 2000). In a similar study 1-methoxycanthinone, isolated from <i>Leitneria floridana</i>, inhibited HIV-1 RT with an IC<sub>50</sub> of 0.26 μg/mL and TI >39 (Xu et al., 2000). Aromoline and FK-3000 isolated from the root tuber of <i>Stephania cephanantha</i>, completely inhibited the cytopathic effects of HIV-1 on MT-4 cells at 31.3 and 7.8 μg/mL, and possessed a CC<sub>50</sub> of 62.5 and 15.6 μg/mL, respectively (Ma et al., 2002). Likewise, the carbazole alkaloid, siamenol from <i>Murraya siamensis</i>, inhibited HIV-1 induced cytopathic inhibitory activity in an XTT assay.

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**Table 1. Terpenoid derived anti-HIV agents**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Source</th>
<th>Part used</th>
<th>Activity/Target</th>
<th>Potency</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Litseagermacrane, 5-epi-eudesm-4(15)-ene-1β,6β-diol and litseachromolaevanes B (sesquiterpenes)</td>
<td><em>Litsea verticillata</em></td>
<td>Leaves and twigs</td>
<td>Inhibited HIV-1 replication</td>
<td>IC_{50} values: 27.5, 73.1 and 119.7 µM, respectively</td>
<td>Zhang et al., 2003a</td>
</tr>
<tr>
<td>Dehydrooxoperezinone (sesquiterpene quinone)</td>
<td><em>Aristolochia manshuriensis</em></td>
<td>Stem</td>
<td>Inhibited HIV-1 replication</td>
<td>EC_{50} = 17.5 µg/mL and TI = 1.43</td>
<td>Wu et al., 2003b</td>
</tr>
<tr>
<td>Ovatodiolide (a diterpenoid)</td>
<td><em>Anisomeles indica</em></td>
<td>Leaves</td>
<td>Inhibited the cytoprotic effects of HIV-1 infection</td>
<td>EC_{50} = 0.10 µg/mL; IC_{50} = 1.20 µg/mL; Maximum cellular protection of 80%–90%</td>
<td>Shahidul Alam et al., 2000</td>
</tr>
<tr>
<td>Agastanol and agastaquinone (diterpenoids)</td>
<td><em>Agastache rugosa</em></td>
<td>Roots</td>
<td>HIV-1 PR</td>
<td>IC_{50} = 360 and 87 µM, respectively</td>
<td>Min et al., 1999a</td>
</tr>
<tr>
<td>Uvaol and ursolic acid (triterpenes)</td>
<td><em>Crateagus pinnatifida</em></td>
<td>Leaves</td>
<td>HIV-1 PR</td>
<td>IC_{50} = 5.5 and 8.0 µM, respectively</td>
<td>Min et al., 1999b</td>
</tr>
<tr>
<td>Garciosaterpene A (24) and C (25) (triterpenes)</td>
<td><em>Garcinia speciosa</em></td>
<td>Bark and stems</td>
<td>HIV-1 RT</td>
<td>IC_{50} = 15.5 and 12.2 µg/mL, respectively</td>
<td>Rukachaisirikul et al., 2003</td>
</tr>
<tr>
<td>Vaticinone (triterpenoid)</td>
<td><em>Vatica cinerea</em></td>
<td>Leaves and stem</td>
<td>Inhibition in syncytium assay</td>
<td>EC_{50} = 5.8 µg/mL with TI 3.4, and 37.0 µg/mL with TI 1.9, respectively</td>
<td></td>
</tr>
<tr>
<td>Moronic acid (triterpenoid)</td>
<td>Propolis</td>
<td>-</td>
<td>Inhibited HIV in H9 cells</td>
<td>EC_{50} = &lt;0.1 µg/mL with TI &gt; 186</td>
<td>Ito et al., 2001</td>
</tr>
<tr>
<td>Lancilactones C (a triterpene lactone)</td>
<td><em>Kadsura lancilimba</em></td>
<td>Stems and roots</td>
<td>Inhibited HIV replication</td>
<td>EC_{50} = 1.4 µg/mL and TI &gt; 71.4</td>
<td>Chen et al., 1999</td>
</tr>
<tr>
<td>Argamine C (a saponin)</td>
<td><em>Tieghemella heckeli</em></td>
<td>Fruits</td>
<td>Inhibited HIV-1 syncytium formation</td>
<td>EC_{50} = 7 µM; EC_{100} = 20 µM; IC_{50} &gt; 20 µM; and TI &gt; 2.85</td>
<td>Gosse et al., 2002</td>
</tr>
<tr>
<td>Actein (a T-type saponin)</td>
<td><em>Cimicifuga racemosa</em> (blackcohosh)</td>
<td>Rhizome</td>
<td>Inhibited HIV-1 replication</td>
<td>EC_{50} = 0.375 mg/mL and TI = 144</td>
<td>Sakurai et al., 2004</td>
</tr>
<tr>
<td>Valtrate (an iridoid)</td>
<td><em>Valerianae Tauriei</em></td>
<td>Roots</td>
<td>Inhibited Rev-mediated nuclear transport</td>
<td></td>
<td>Murakami et al., 2002</td>
</tr>
<tr>
<td>Pheophorbide A (a chlorophyll isolate)</td>
<td><em>Vatica cinerea</em></td>
<td>Leaves and stem</td>
<td>Inhibited HIV-1 replication</td>
<td>IC_{50} = 1.5 µg/mL (25 µM) TI &gt; 13</td>
<td>Zhang et al., 2003b</td>
</tr>
</tbody>
</table>

*a* HIV-1 protease; *b* HIV-1 reverse transcriptase.
with an EC$_{50}$ of 2.6 µg/mL and TI of 2.4 (Meragelman et al., 2000).

The methanol extract of Artemisia caruolia yielded N$^2$,N$^5$,N$^{10}$-tri-p-coumaroyspermidine which showed a moderate inhibitory activity on HIV-1 protease (EC$_{50}$ = 53 µg/mL). Based on the lead structure two related amides, namely, N$^2$,N$^5$,N$^{10}$,N$^{14}$-tetra-p-coumaroyspermine and N$^2$,N$^5$,N$^{10}$,N$^{15}$-penta-p-coumaroyltaetraethylenepentamine, were then synthesized and inhibited HIV-1 protease more potently and N$^2$,N$^5$,N$^{10}$-tri-p-coumaroyspermidine, with EC$_{50}$ values of 27 and 30 µg/mL, respectively (Ma et al., 2001).

### POLYPHENOLS

Chronic administration of polyphenol-rich fruit juices is thought to be favourable to HIV-positive patients due to enhanced phytohaemagglutinin-induced lymphocyte proliferation, which could restore disturbances in T-cell homeostasis. Apoptosis that counterbalances increased lymphocyte proliferation in healthy individuals during juice consumption is absent in the case of HIV patients (Winkler et al., 2004).

The gallotannins geraniin (32) and corilagin (33) isolated from Phyllanthus amarus demonstrated a specific inhibition of HIV-1 replication in MT-4 cells with an EC$_{50}$ of 0.24 µg/mL and TI values of 26.8 and 29.3, respectively. Geraniin was shown to be active at two distinct sites of the HIV replication, which is helpful in suppressing the emergence of escape mutants. It effectively blocked viral uptake (EC$_{50}$ < 2.5 µg/mL) and also exhibited in vitro inhibition of HIV-1 RT at an IC$_{50}$ of 1.9 µM, a potency about 1000 fold higher than AZT-TP. It seems that this has enabled it to inhibit various nucleoside reverse transcriptase inhibitor (NRTI) and NNRTI resistant HIV-1 and HIV-2 strains, making it a good candidate for salvage therapy. Geraniin’s activity against RT differs from the approved RT inhibitors by not being competitive with respect to the primer/template and it has been proposed that the galloyl products. Table 2 summarizes other anti-HIV polyphenol natural compounds that demonstrated sound anti-HIV activities without showing cytotoxicity to H9 cells at high concentrations (CC$_{50}$ > 297 µM and >223 µM, respectively). Both inhibitors strongly suppressed the acute HIV-1 infection of H9 cells with IC$_{50}$ values of 2 and 6.9 µM, respectively. These compounds inhibited two HIV-1 integrase activities, 3'-processing and 3'-joining to the 5'-ends of the target DNA with IC$_{50}$ values in the range 0.37–0.83 µM. Both compounds neither prevent HIV entry into H9 cells nor inhibit RT activity in infected cells. These two selective integrase inhibitors hold promise as a novel class of therapeutic drugs for AIDS based on their high potencies and absence of cytotoxicity (Abd-Elazem et al., 2002). In relation to this, rosmarinic acid methyl ester and rosmanarinic acid, isolated from Coleus parvifolius (Labiateae), inhibited HIV-1 integrase with IC$_{50}$ values of 3.1 and 5.0 µM, respectively (Kim et al., 1999; Tewtrakul et al., 2003). The HIV-1 integrase inhibitory effects of rosmarinic acid derivatives increase in order of monomers, dimers (IC$_{50}$ = 5.0 µM), trimers, e.g. lithospermic acid (IC$_{50}$ = 1.4 µM) and tetramers, e.g. lithospermic acid B (IC$_{50}$ = 1.0 µM). It was shown that the metal-chelating derivatives were more potent than those that are non-binding (Tewtrakul et al., 2003).

Polyphehols from Prunella vulgaris and Rhizoma cibotte potently blocked gp41 six-helix bundle formation (a critical step of HIV-1 fusion with target cells) in a similar way to tannin; 86.2% and 98.3% at 50 µg/mL, respectively. Tannin and other polyphenols may be developed as a topical microbicide for the prevention of sexual transmission of HIV (Liu et al., 2002).

St. John’s worth potently inhibits UV-induced activation of HIV gene expression in stably transfected HIVcat/HeLa cells, in a dose-dependent manner. Since hypericin is known to exhibit a similar inhibitory property, it is likely to be the active constituent of St. John’s work (Tahir et al., 2002).

Polycaphenol, the alkaline extract of cacao husk, effectively inhibited the cytotoxic effect of HIV infection in MT-4 cells and also protected mice from lethal infection of E. coli. In addition to this, polycaphenol exhibits selective antitumour activities against human oral tumour cells such as human oral squamous cell carcinoma (HSC-2) and human salivary gland tumour (HSG) and also possesses antioxidant properties (Jiang et al., 2001). Thus polycaphenol could be of an immense significance in the management of chronic HIV/AIDS. Table 2 summarizes other anti-HIV polyphenol natural products.

### POLYSACCHARIDES

Sulphated polysaccharides of marine origin have been reviewed comprehensively by De Clercq (2000). Sulphated polysaccharides block the binding (adsorption) of retroviruses to cells, which, in the case of HIV, is due to a direct interaction of the sulphated polysaccharides with the v3 loop of the viral envelope gp120. As a consequence, sulphated polysaccharides also block syncytium formation (fusion) between HIV-infected cells expressing the gp120 glycoprotein on their surface and uninfected cells expressing the CD4 receptor for gp120 (Baba et al., 1990). Sulphated
Table 2. Polyphenol derivatives with anti-HIV activities

<table>
<thead>
<tr>
<th>Source</th>
<th>Protein</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; µg/mL</th>
<th>HIV-1 IN&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HIV-1 PR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Syncytia formation</th>
<th>HIV-1 INHIBITION</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clerodendron trichotomum</td>
<td>Acteoside</td>
<td>7.8 ± 1.96 µg/mL</td>
<td>16.1 ± 1.96 µg/mL</td>
<td>7.0 ± 1.96 µg/mL</td>
<td>68.2% inhibition</td>
<td>0.9 mg/mL</td>
<td>Kim et al., 2000</td>
</tr>
<tr>
<td>Aster scaber</td>
<td>and acteoside isomer</td>
<td>13.7 ± 1.96 µg/mL</td>
<td>7.8 ± 1.96 µg/mL</td>
<td>7.0 ± 1.96 µg/mL</td>
<td></td>
<td></td>
<td>et al., 2001a</td>
</tr>
<tr>
<td>Maytenus senegalensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camellia japonica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kwon et al., 2000</td>
</tr>
<tr>
<td>Eugenia caryophyllata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Hussein et al., 2001)</td>
</tr>
<tr>
<td>Fraxinus sieboldiana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a HIV-1 integrase; b HIV-1 protease.

Terrestrial non-sulphated polysaccharides also have their share in the list of anti-HIV natural products. A polysaccharide extracted from the leaf of *Rhizophora apiculata* (RAP) inhibited HIV-1, HIV-2 and SIV strains in MT-4, PBMC and MAGI-CCR5 cells in various assay systems including inhibition of viral cytopathogenicity by the MTT assay, antigen expression, p24 production, or the MAGI assay, within an EC<sub>50</sub> concentration range of 6.5 to 40.6 µg/mL. For instance, it blocked the expression of HIV-1 antigen in MT-4 cells and abolished the production of HIV-1 p24 antigen in PBMC with EC<sub>50</sub> values of 10.7 (TI = 144.5) and 25.9 µg/mL (TI = 44.0), respectively. RAP, at 100 µg/mL, completely blocked the binding of HIV-1 virions to MT-4 cells. RAP also reduced the production of viral mRNA when added before virus adsorption. It inhibited syncytium formation in cocultures of MOLT-4 cells and MOLT-4/ HIV-1(IIIB) cells (EC<sub>50</sub> values 53.3 µg/mL); gp120 seemed to be its target. These properties may be advantageous should RAP be considered for further development as a vaginal anti-HIV formulation. Moreover, RAP did not prolong activated partial thromboplastin time (APTT) up to 500 µg/mL. The acid polysaccharide RAP had a molecular weight of more than 30 000 and was mainly composed of galactose, galactosamine and uronic acid. Its antiviral activity may be attributed to its carboxylated (polyamionic) character (Premanathan et al., 1999a). A similar anti-HIV polysaccharide extracted from the bark of *Rhizophora mucronata* has also been characterized by Premanathan et al. (1999b).

Various sulphated polysaccharides are now being tested for their clinical efficacy. The experience with topically applied dextrin sulphate in human subjects (both male and female) shows that sulphated polysaccharides do not produce systemic toxicity or genital epithelial disruption (Low-Beer et al., 2002; Van Damme et al., 2002). Another sulphated polymannuroguluronate (SPMG), a marine sulphated polysaccharide extracted from brown algae with specific means of fractionation and chemical modification, has entered Phase II clinical trial in China as the first anti-AIDS drug candidate obtained from marine organisms (Miao et al., 2004). SPMG exhibits a significant inhibitory effect against HIV proliferation in both normal human umbilical vein endothelial cells (HUVEC) and bFGF-treated HUVEC (Wang et al., 2003).

### PROTEINS

This is the largest group of natural products with anti-HIV activity. The botanical sources, size and potency of most of these compounds are summarized in Table 3 and those which are considered to be most important are discussed below.

Cyanovirin-N (CV-N) (38) is a 101 residue (11 kDa) protein originally isolated from the blue-green alga...
<table>
<thead>
<tr>
<th>Protein</th>
<th>Source</th>
<th>Part of plant</th>
<th>Size (kDa)</th>
<th>Target</th>
<th>Potency (IC₅₀)</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angularin</td>
<td><em>Vigna angularis</em> (adzuki bean)</td>
<td>Seeds</td>
<td>8</td>
<td>HIV-1 RT*</td>
<td>27.5% at 70 μM</td>
<td>• Antifungal</td>
<td>Ye and Ng, 2002c</td>
</tr>
<tr>
<td>Ascalin</td>
<td><em>Allium ascalonicum</em></td>
<td>Bulbs</td>
<td>9.5</td>
<td>HIV-1 RT</td>
<td>10 μM</td>
<td>• Antifungal</td>
<td>Wang and Ng, 2002b</td>
</tr>
<tr>
<td>α-Basrubrin</td>
<td><em>Basella rubra</em> (Ceylon spinach)</td>
<td>Seeds</td>
<td>4.3</td>
<td>HIV-1 RT</td>
<td>79.4% and 10.56% at 400 and 40 μM respectively</td>
<td>• Inhibit translation in RRLS</td>
<td>Wang and Ng, 2001a</td>
</tr>
<tr>
<td>β-Basrubrin</td>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>54.6% and 2.12% at 400 and 40 μM respectively</td>
<td>• Inhibit translation in RRLS</td>
<td></td>
</tr>
<tr>
<td>Castanopsis thaumatin-like protein</td>
<td><em>Castanopsis chinensis</em></td>
<td>Seeds</td>
<td>30</td>
<td>HIV-1 RT</td>
<td>1.6 μM</td>
<td>• Antifungal</td>
<td>Chu and Ng, 2003b</td>
</tr>
<tr>
<td>Chickpea cyclophilin-like antifungal protein</td>
<td><em>Cicer arietinum</em> (chickpea)</td>
<td>Seeds</td>
<td>18</td>
<td>HIV-1 RT</td>
<td>69.8% at 27.7 μM and 97.8% at 277.7 μM</td>
<td>• Antifungal; Weakly inhibited cell-free translation</td>
<td>Ye and Ng, 2002e</td>
</tr>
<tr>
<td>Chrysancorin</td>
<td><em>Chrysanthemum coronarium var. spatiosum</em></td>
<td>Seeds</td>
<td>13.4</td>
<td>HIV-1 RT</td>
<td>84.56% and 0.0% at 22% 2.2 μM</td>
<td>• Antifungal; Mitogenic in MSC</td>
<td>Wang et al., 2001</td>
</tr>
<tr>
<td>Contrajervin</td>
<td><em>Dorstenia contrajerva</em></td>
<td>Leaves</td>
<td>5</td>
<td>Bind to gp41 BIND GP120 Inhibited HIV-1 induced cytopathogenicity</td>
<td>EC₅₀ = 1.0 μM and IC₅₀ &gt; 4.9 μM</td>
<td>• Antifungal; Mitogenic in RLS</td>
<td>Bokesch et al., 2004</td>
</tr>
<tr>
<td>Cowpea α-antifungal protein</td>
<td><em>Vigna unguiculata</em> (Cowpea)</td>
<td>Seeds</td>
<td>28</td>
<td>HIV-1 RT α-Glucosidase</td>
<td>54.3% at 5 mg/mL</td>
<td>• Antifungal; Inhibit translation in RLS</td>
<td>Ye et al., 2000</td>
</tr>
<tr>
<td>Cowpea β-antifungal protein</td>
<td><em>V. unguiculata</em></td>
<td>Seeds</td>
<td>12</td>
<td>HIV-1 RT β-Glucosidase</td>
<td>66.4% at 5 mg/mL</td>
<td>• Antifungal; Inhibit translation in RLS</td>
<td></td>
</tr>
<tr>
<td>Delandin</td>
<td><em>Delandia unbellata</em> (rice bean)</td>
<td>Seeds</td>
<td>28</td>
<td>HIV-1 RT</td>
<td>44.5%, 32%, 13.4% at 180, 18, 1.8 μM, respectively</td>
<td>• Antifungal; Inhibit translation in RLS; Mitogenic in MSC</td>
<td>Ye and Ng, 2002d</td>
</tr>
<tr>
<td>Fabin</td>
<td><em>Vicia faba</em> (broad bean)</td>
<td>Seeds</td>
<td>34</td>
<td>HIV-1 RT</td>
<td>34 μM</td>
<td>• Antifungal</td>
<td>Ng and Ye, 2003</td>
</tr>
<tr>
<td>Ginkbilobin</td>
<td><em>Ginkgo biloba</em></td>
<td>Seeds</td>
<td>13</td>
<td>HIV-1 RT</td>
<td>75.1% at 2 mg/mL</td>
<td>• Antifungal; Antibacterial; Anti-mitogenic in MSC</td>
<td>Wang and Ng, 2000b</td>
</tr>
<tr>
<td>Ground bean lectin</td>
<td><em>Vigna sesquipedalis</em> (ground bean)</td>
<td>Seeds</td>
<td>−60</td>
<td>HIV-1 RT</td>
<td>73 μM</td>
<td>• Hemagglutinating activity inhibited by polygalacturonic acid but not galacturonic acid and simple monosaccharides; Decreased viability of hepatoma (HepG2), leukaemia (L1210), and leukaemia (M1) cells; Mitogenic in MSC</td>
<td>Wong and Ng, 2003</td>
</tr>
<tr>
<td>A homodimeric lectin</td>
<td><em>Phaseolus vulgaris</em> (red kidney beans)</td>
<td>Seeds</td>
<td>67</td>
<td>HIV-1 RT and α-glucosidase</td>
<td>80.2% at 5 mg/mL</td>
<td>• Antifungal</td>
<td>Ye et al., 2001b</td>
</tr>
<tr>
<td>Protein</td>
<td>Source</td>
<td>Part of plant</td>
<td>Size (kDa)</td>
<td>Target</td>
<td>Potency (IC&lt;sub&gt;50&lt;/sub&gt;)</td>
<td>Remarks</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>---------------------------------------------</td>
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<td>------------</td>
<td>-------------------------</td>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Hypoginin</td>
<td><em>Arachis hypogaea</em> (peanut)</td>
<td>Seeds</td>
<td>7.2</td>
<td>HIV-1 RT</td>
<td>58.9% at 5 mg/mL</td>
<td>• Antifungal</td>
<td>Ye and Ng, 2001b</td>
</tr>
<tr>
<td>Kiwi fruit thaumatin-like protein</td>
<td><em>Actinidia chinensis</em></td>
<td>Fruits</td>
<td>21</td>
<td>HIV-1 RT</td>
<td>30.6% at 27 µM</td>
<td>• Antifungal</td>
<td>Wang and Ng, 2002a</td>
</tr>
<tr>
<td>A laccase</td>
<td><em>Tricholoma giganteum</em></td>
<td>Fresh fruits</td>
<td>43</td>
<td>HIV-1 RT</td>
<td>2.2 µM</td>
<td>• Antifungal</td>
<td>Wang and Ng, 2004</td>
</tr>
<tr>
<td>Lentin</td>
<td><em>Lentinus edodes</em> (shiitake mushroom)</td>
<td>Fruiting bods</td>
<td>27.5</td>
<td>HIV-1 RT</td>
<td>1.5 µM</td>
<td>• Antifungal</td>
<td>Ngai and Ng, 2003</td>
</tr>
<tr>
<td>Lilin</td>
<td><em>Lilium brownii</em></td>
<td>Bulbs</td>
<td>14.4</td>
<td>HIV-1 RT</td>
<td>97.93% at 120 µM</td>
<td>• Antifungal</td>
<td>Wang and Ng, 2002c</td>
</tr>
<tr>
<td>Lyophyllin</td>
<td><em>Lyophyllum shimeji</em></td>
<td>Fruiting bods</td>
<td>20</td>
<td>HIV-1 RT</td>
<td>7.9 nM</td>
<td>• An RIP&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Lam and Ng, 2001a</td>
</tr>
<tr>
<td>Lyophyllin antifungal protein</td>
<td><em>Lyophyllum shimeji</em></td>
<td>Fruiting bods</td>
<td>14</td>
<td>HIV-1 RT</td>
<td>5.2 nM</td>
<td>• Antifungal</td>
<td>Lam and Ng, 2001b</td>
</tr>
<tr>
<td>A mannose-binding lectin</td>
<td><em>Allium tuberosum</em></td>
<td>Inner shoots</td>
<td>13</td>
<td>HIV-1 RT</td>
<td>67.7% at 0.1 mg/mL</td>
<td>• Mitogenic in MSC</td>
<td>Lam and Ng, 2001b</td>
</tr>
<tr>
<td>Mollisin</td>
<td><em>Castanea mollisima</em></td>
<td>Seeds</td>
<td>28</td>
<td>HIV-1 RT</td>
<td>14 µM</td>
<td>Antifungal</td>
<td>Chu and Ng, 2003a</td>
</tr>
<tr>
<td>Pananotin</td>
<td><em>Panax notoginseng</em> (sanchi ginseng)</td>
<td>Root</td>
<td>35</td>
<td>HIV-1 RT</td>
<td>35.8% at 12.6 µM and 24.7% at 1.26 µM</td>
<td>• Antifungal</td>
<td>Lam and Ng, 2002a</td>
</tr>
<tr>
<td>Phasein A</td>
<td><em>Phaseolus vulgaris</em> (pinto beans)</td>
<td>Seeds</td>
<td>28</td>
<td>HIV-1 RT</td>
<td>29 µM</td>
<td>• Antifungal</td>
<td>Ye and Ng, 2002f</td>
</tr>
<tr>
<td>Phasein B</td>
<td></td>
<td></td>
<td>32</td>
<td>HIV-1 RT</td>
<td>8 µM</td>
<td>• Inhibit translation in RRLS</td>
<td></td>
</tr>
<tr>
<td>Quinqueginsin</td>
<td><em>Panax quinquefolium</em> (American ginseng)</td>
<td>Roots</td>
<td>53</td>
<td>HIV-1 RT</td>
<td>Not determined</td>
<td>• Anti HIV-1 RT activity potentiated after chemical modification with succinic anhydride</td>
<td>Wang and Ng, 2000a</td>
</tr>
<tr>
<td>Rice bean antifungal peptide</td>
<td><em>Delandia unbellata</em> (rice bean)</td>
<td>Seeds</td>
<td>5</td>
<td>HIV-1 RT</td>
<td>39.0% at 1.02 nm and 24.1% at 0.10 nm</td>
<td>• Antifungal</td>
<td>Ye and Ng, 2002b</td>
</tr>
<tr>
<td>Treculavir</td>
<td><em>Treculia obovoidea</em></td>
<td>Bark</td>
<td>10</td>
<td>Inhibited HIV-1 induced cytopathogenicity Bind to gp41</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; &lt; 0.02 µM and IC&lt;sub&gt;50&lt;/sub&gt; &gt; 2.5 µM and 0.20 µM</td>
<td>• Antifungal</td>
<td>Bokesch et al., 2004</td>
</tr>
<tr>
<td>A trypsin-chymotrypsin inhibitor peptide</td>
<td><em>Vicia faba</em> (broad bean)</td>
<td>Seeds</td>
<td>13</td>
<td>HIV-1 RT</td>
<td>32 µM</td>
<td>• Antifungal</td>
<td>Ye and Ng, 2002a</td>
</tr>
</tbody>
</table>
NATURALLY DERIVED ANTI-HIV AGENTS

CV-N is well tolerated by human cells (CEM-SS and PBL) at high concentrations (e.g., 9000 nM). The binding event (Han et al., 2001; Otero-Quintero, 2001; Bewley et al., 2001; Mariner et al., 2000; Esser et al., 1999; Mariner et al., 1998; Mori and Boyd, 2001; O’Keefe et al., 2000).

1. CV-N impaired the binding of virion-associated gp120 to cell-associated CD4.
2. CV-N preferentially inhibited binding of the glycosylation-dependent neutralizing monoclonal antibody 2G12 (MAb 2G12) to gp120. However, MAb 2G12 pretreatment did not prevent subsequent CV-N binding to soluble gp120 (sgp120).
3. CV-N did not interfere with the interactions of soluble CD4 (sCD4) with either sgp120 or virion-associated gp120.
4. Prebinding of sgp120 to sCD4 did not block the subsequent binding of CV-N with the sgp120.
5. CV-N impairs both CD4-dependent and CD4-independent binding of sgp120 to the target cells. CV-N also impairs interaction of sCD4-activated Env with the coreceptor CCR5.
6. CV-N blocks the sCD4-induced binding of sgp120 with cell-associated coreceptor CXCR4.
7. CV-N dissociates bound sgp120 from target cells.
8. Pretreatment of CV-N with either sgp120 or sgp41 abrogated the neutralizing activity of CV-N against intact infectious HIV-1 virions.

CV-N–gp120 interactions are in part mediated by N-linked complex carbohydrates present on gp120, i.e., carbohydrate-dependent interaction. CV-N can exist in solution either as a monomer or a dimer. In the monomer form it contains a novel carbohydrate bind-
biological activity of CV-N is extremely resistant to physiochemical degradation and can withstand treatment with denaturants, detergents, organic solvents, multiple freeze-thaw cycles and heat (up to 100 °C) with no apparent loss of antiviral activity (Boyd et al., 1997). CV-N is further pursued as a topical (vaginal or rectal) microbicide to prevent sexual transmission of HIV, and preclinical development is under investigation. Efforts are also underway to produce its functional homologues in prokaryotic and eukaryotic hosts, which would enable their large-scale production (Boyd et al., 1997; Mori et al., 1998; Mori et al., 2002). CV-N could also be expressed as surface protein as well as secreted as soluble protein by the human commensal bacterium Streptococcus gordonii providing an alternative means to deliver and maintain an effective concentration of the microbicide in the vaginal mucosa (Giomarelli et al., 2002). Moreover, by coupling it with Pseudomonas exotoxin, a conjugate molecule capable of selectively killing HIV-infected gp120-expressing cells has been produced (Mori et al., 1997).

Pf-gp6, a 6 kDa anti-degranulation glycoprotein purified from the extract of Perilla frutescens inhibited HIV-1-induced cytopathic effect and proviral DNA synthesis. Its IC50 for various HIV-1 strains, including clinical isolates and CCR5-using (R5) HIV-1, ranged between 1.3 and 71.0 µg/mL, depending on the combination of viral strain and host cell. It also inhibited HIV-2_Rod in MT-4 with an IC50 of 7.8 µg/mL. Pf-gp6 did not directly inactivate infectious viral particles. A time-of-addition experiment revealed that Pf-gp6 lost its activity before zidovudine but after the CXCR-4 antagonist AMD3100 during the early stage of viral infection. Although the pinpoint target of Pf-gp6 remains to be elucidated, it may interfere with a step between viral entry and reverse transcription (Kawahata et al., 2002).

A comparative study of a variety of antifungal proteins from the seeds of leguminous plants including French bean, cowpea, field bean, mung bean, peanut and red kidney bean, show that nearly all proteins examined were able to inhibit HIV-1 RT, protease and integrase enzymes, to different extents (Ng et al., 2002).

Pokeweed antiviral proteins (PAPs) are related broad-spectrum antiviral proteins isolated from the leaves of the pokeweed plant, Phytolacca americana. They are single-chain ribosome-inactivating proteins that catalytically depurinate ribosomal as well as viral RNA including that of HIV-1. There are three well-known different pokeweed antiviral protein (PAP) isoforms, namely. PAP-I from the spring leaves, PAP-II from the early summer leaves and PAP-III from the late summer leaves. PAP-I, PAP-II and PAP-III effectively inhibit the replication of HIV-1_HTLV-IIIb in human peripheral blood mononuclear cells at concentrations which do not inhibit the protein synthesis of host cells; IC50 values; 17 nm, 25 nm and 16 nm, respectively (Irvin and Uckun, 1992; Rajamohan et al., 1999a; Rajamohan et al., 1999b).

Depurination alone did not account for the high potency of PAP against HIV-1 and its mechanism is not yet fully understood. Molecular modelling studies predicted a more potent anti-HIV activity for PAP-III due to its unique surface topology and more favourable charge distribution in its 20 Å-long RNA binding active centre cleft. In accordance with this prediction, PAP-III was more potent than PAP-I in depurinating HIV-1 RNA. Residues Tyr(69), Tyr(117), Gln(172) and Arg(175) are expected to define the active site of PAP-III (Rajamohan et al., 1999b; Kurinov and Uckun, 2003).

A molecular model of PAP–RNA interactions was used to rationally engineer FLP-102(28.6 kD) isolated from the ripe fruit and seeds. MRK29 might also have a modulatory role on HIV-1. PAP is further pursued as a nonspermicidal microbicide (D’Cruz et al., 2001a; D’Cruz and Uckun, 2001b; D’Cruz et al., 2004a). Findings of a recent study, however, indicate that careful monitoring of vaginal irritation might be required in the clinical development of PAP as a nonspermicidal microbicide (D’Cruz et al., 2004b).

The clinical use of native PAP is limited due to inherent difficulties in obtaining sufficient quantities of a homogenously pure and active PAP preparation with minimal batch to batch variability from its natural source. However, it has been shown that PAP can be produced by DNA recombination in E. coli (Rajamohan et al., 1999c) and Pichia pastoris (Rajamohan et al., 2000).

A number of anti-HIV proteins have been isolated from Thai bitter gourd, i.e. Momordica charantia. Among these is the 20 amino acid protein MRK29 (28.6 kD) isolated from the ripe fruit and seeds. MRK29 inhibited the HIV-1 RT with an IC50 of 18 µg/mL. It also exerted 82% reduction of viral replication at 0.175 µg/mL in HIV-infected cells. It is postulated that MRK29 might also have a modulatory role on immune cells, because it increased 3-fold TNF activity (Jiratchariyakul et al., 2001). In addition to this, a ribosome inactivating protein (RIP) designated MAP30
(Momordica anti-HIV protein, 30 kDa) acts as a DNA glycosylase/apurinic lyase, and was therefore able to inhibit HIV-1 integrase and irreversibly relax supercoiled DNA. The toxicity of MAP30 is specific to tumour-transformed or viral-infected cells. It shows no adverse effects on normal cells since it can not penetrate them (Lee-Huang et al., 1990; Lee-Huang et al., 1995; Wang et al., 1999). Furthermore, MAP30 inhibits the proliferation of BC-2, an AIDS-related primary effusion lymphoma (PEL) cell, latently infected with Kaposi’s sarcoma-associated herpesvirus (KSHV), also known as human herpes virus 8 (HHV8). MAP30 modulates the expression of both viral and cellular genes involved in Kaposi’s sarcoma pathogenesis. It downregulates the expression of viral cyclin D (vCD), viral interleukin-6 (vIL-6) and viral FLIP (vFLIP), genes involved in cell cycle regulation, viral pathogenesis and apoptosis. MAP30 also downregulates the expression of various cellular genes involved in mitogenesis, tumorigenesis and inhibition of apoptosis in NFkB and p53 signalling pathways, while it upregulates the pro-apoptotic-related genes Bax, CRADD and caspase-3 (Sun et al., 2001). RIPs in general, and MAP30 in particular, hold potential as both anti-tumour and anti-HIV agents. MAP30 may also be useful as a nonspermicidal prophylactic against sexual transmission of HIV since it did not alter the viability and motility of human spermatozoa even at a dose 1000 times the maximum effective concentration that inhibit HIV-1 and herpes simplex virus (Schreiber et al., 1999). Another anti-HIV protein, α-momorcharin from the same plant was reported to possess remarkable anti-HIV-1 properties (Zheng et al., 1999).

α-Trichobitacin, a novel RIP from the root tubers of Trichosanthes kirilowii, exhibited anti-HIV activities virtually similar to those of α-momorcharin. α-Trichobitacin greatly suppressed HIV-1-induced syncytial cell formation (IC50 = 5 µg/L) and markedly reduced both the replication of HIV-1 and the number of HIV antigen positive cells (IC50 = 0.09 mg/L) in acutely but not chronically HIV-1 infected culture (Zheng et al., 2000). Au et al. (2000) reported that inhibition of HIV-1 integrase appears to account partly for the anti-HIV properties of common RIPs.

Palicourea, a 37 amino acid cyclotide isolated from the tropical tree Palicourea condensata, inhibited the in vitro cytopathic effects of HIV-1RF infection of CEM-SS cells with EC50 and IC50 values of 0.1 µm and 1.5 µm, respectively (Bokesch et al., 2001). Hallock et al. (2000) have reported four novel macrocyclic peptides containing 28–31 amino acid residues, named cyclovilions A–D, isolated from the hitherto tropical plant Leonia cymosa (Violaceae) that suppress HIV-1 replication in CEM-SS cells with an EC50 ~0.13 µm. Similarly, four new macrocyclic polypeptides designated circulins C–F, from another tropical tree Chassalia parvifolia, inhibited the cytopathic effects of in vitro HIV-1 infection with EC50 values of 50–275 nm. Circulins C–F are 29–30 amino acid cyclotides (Gustafson et al., 2000).

Mannose-specific lectins from the bulbs of wild Narcissus spp. growing in Spain could suppress HIV-1 infection of MT-4 without significant cytotoxicity. Their haemagglutination and anti-HIV-1 activities showed no significant correlation. Multiple isolate composition has been suggested to account for this dissociation between the two activities (Lopez et al., 2003).

A lectin from Myrianthus holstii designated M. holstii lectin repressed CEM-SS infection by HIV-1RF with an EC50 value of 150 nm. Delaying the addition of the lectin for up to 8 h after initial exposure of CEM-SS cells to virus did not result in a loss of the antiviral activity; however, a 16 h or more delay resulted in a marked decrease in the antiviral activity. The lectin bound to a virus-free, soluble form of the viral envelope protein gp120 but did not inhibit the subsequent binding to a cell-free, soluble form of the cellular receptor CD4 (Charan et al., 2000). Lectins from Phaseolus vulgaris, Momordica charantia, Ricinus communis and Agaricus bisporus were reported to inhibit HIV-1 RT (Wang and Ng, 2001c).

**MISCELLANEOUS**

In an *in vitro* XTT-based anti-HIV assay, 2–5 µg/mL of the polyacetylenic acid, minquartynoic acid [(−)-17-hydroxy-9,11,13,15-octadecatetraynoic acid] from Ochanostachys amentacea, effectively inhibited human lymphoblastoid cell killing by HIV-1 (Rashid et al., 2001).

Olive leaf extract (OLE) inhibits acute infection of MT-2 cells with HIV and cell-to-cell transmission of HIV-1. It also inhibits HIV-1 replication in infected H9 cells. These anti-HIV effects of OLE are dose dependent, with EC50 values of around 0.2 µg/mL. In the effective dose range, no cytotoxicity on uninfected target cells was detected (TI > 5000). HIV-1 infection modulates the expression patterns of cellular genes involved in apoptosis, stress, cytokine, PKC and hedgehog signalling. HIV-1 infection upregulates the expression of the heat-shock PKC proteins hsp27 and hsp90, the DNA damage inducible transcript 1 gadd45, the p53-binding protein mdm2, and the hedgehog signal protein patched 1, while it downregulates the expression of the anti-apoptotic BCL2-associated X protein Bax. Treatment with OLE reverses many of these HIV-1 infection-associated changes. It also upregulates the expression of the apoptosis inhibitor proteins IAP1 and 2, as well as the calcium and PKC pathway signalling molecules IL-2, IL-2Ralph, and ornithine decarboxylase ODC1 (Lee-Huang et al., 2003).

The aqueous extracts of Ocimum gratissimum (leaves), Ficus polita (leaves), Clausena anisata (leaves), Alchornea cordifolia (fruits and seeds) and Elaeophorbia drupifera (leaves) are effective inhibitors of HIV-1 and HIV-2 replication. Most of the plant extracts inhibited HIV-1<sub>HTLVIII</sub> cytopathicity with EC50 values in the range of 0.01 to 0.03 mg/mL, and TI values, 18 to 110. The leaves of *O. gratissimum* and the seeds of *A. cordifolia* had the highest TIs; 110 and 90, respectively. Except those of *A. cordifolia*, the other plant extracts inhibited HIV-2 strain GHI replication in Molt-4 clone 8 cells with EC50 values in the range <0.005–0.110 mg/mL, and TI values, 12.7–260. Pertaining to anti-HIV-2 activity *F. polita* showed the highest activity accompanied with the highest selectivity: EC50 < 0.005 mg/mL and TI > 260. The plant extracts, unlike AZT, were able to achieve significant inhibition of viral cytopathicity even at high MOI when treatment was delayed for 2 h. Early fusion of chronically HIV-infected cells with uninfected cells has been shown...
to be inhibited by all the plant extracts. In addition, the extract of *E. drupifera* has been identified to be selectively toxic to chronically infected cells at concentrations that are not toxic to uninfected cells. *O. grattissimum, F. polita* and *A. cordifolia* (fruits) caused 90% reduction in HIV-1 RT activity at concentrations between 0.013 and 0.020 mg/mL. HIV-1 proviral DNA copying as determined in a polymerase chain reaction, was completely inhibited by *O. grattissimum* and *F. polita* at 0.011 and 0.015 mg/mL, respectively. The aforementioned plants thus appear to be promising sources for new antiretroviral compounds (Ayisi and Nyadedzor, 2003).

A polyherbal preparation, designated Immu-25, was evaluated for clinical efficacy and safety in HIV-infected patients, with confirmed HIV infection and a CD4 count <500 cells/µL. The polyherbal test preparation produced good symptomatic improvement within 6 months. The incidence and severity of symptoms such as diarrhoea, fatigue, anorexia, cough and fever decreased with drug treatment. There was a significant decrease in the mean viral load. The decrease in viral load was associated with an increase in mean CD4 count. With the exception of mild gastrointestinal adverse effects, the drug was well tolerated. Both patients and investigators rated the treatment as good or very good. It has been suggested that this herbal drug may have a good immunomodulatory effect and has potential as a co-therapeutic agent in the management of HIV infection (Usha et al., 2003).

*Momordica charantia* (Thai bitter gourd) is a popular medicinal plant that is used for the treatment of various diseases. Among others it has antiviral, antitumour and immune system boosting properties. Chronic administration of a combination of juices and decoction of the leaves and fruits of Thai bitter gourd is reported to have increased the CD4 count and later normalized the CD4/CD8 ratios in an HIV-infected man in California (Rebultan, 1995).

CD4+ T cell counts in HIV-1-infected patients treated with only Korean red ginseng (KRG) were maintained or even increased for a prolonged period and also the development of resistance mutations in RT to zidovudine (ZDV) was delayed by combined therapy with KRG and ZDV. It is suggested that the maintenance of CD4+ T cell counts by ZDV and KRG intake for a prolonged period might be indirectly associated with delayed development of resistance to ZDV by KRG intake (Cho et al., 2001).

It has been reported that a component of garlic called ajoene protects CD+ cells from attack by HIV early in the viral life cycle. At low concentrations, the drug appears to have little toxicity, and its anti-HIV activity is 45 times more powerful than dextran sulphate. Ajoene is found only in fresh garlic and is not readily available. It has been found that garlic impairs the activity of the liver enzymes that process protease inhibitors and raises the protease inhibitor levels (Anonymous, 1998).

Bioavailability of the protease inhibitor, saquinavir, was said to increase with consumption of grapefruit juice, while its clearance was not affected. Inhibition of cytochrome P450 3A4, an intestinal and liver enzyme, which breaks down saquinavir, was suggested to be responsible for this observation (Kupferschmidt et al., 1998). However, in a similar type of study, it was found that concomitant administration of grapefruit juice increases gastric pH and delayed indinavir absorption but did not uniformly affect the systemic bioavailability of indinavir in HIV-infected subjects (Shelton et al., 2001).

European mistletoe (*Viscum album*) has been used parenterally for more than 80 years as an anticancer medication with significant immunomodulating action. Since 1984, clinical experience with a *Viscum album* extract (*Viscum album* Quercus Frischsaft) among HIV-positive patients has suggested that it inhibits HIV disease progression (Gorter et al., 1999). Table 4 summarizes anti-HIV medicinal plant extracts with unidentified constituents.

### CONCLUSION

Next to malaria, acquired immunodefi ciency syndrome (AIDS) is the leading infectious cause of death in the world. Untreated disease caused by the human immunodefi ciency virus (HIV) has a case fatality rate that approaches 100%. Up to now, the complete suppression of HIV replication in patients has not been more than a wildly ambitious idea. Even though antiretroviral drugs have been discovered that can bring about the suppression of the serum load of the virus to undetectable levels, economical, commercial and political barriers have limited their accessibility to a good part of the population suffering from the disease, which indisputably is found in the developing countries. The emergence of resistance and adverse reactions have also limited the utility of many of the conventional antiretroviral drugs. Patient compliance is also one of the challenges in combating the disease. The impact that the progress of HIV/AIDS has in developing countries is multidimensional and most of all is a vicious circle. Needless to stress that there is an urgent need for a rapid and sustainable solution that must be functional in the developing countries. One of the sources for this kind of solution is the rich wealth of medicinal plants that many developing countries are endowed with. As long as their use is backed up with scientific proof, it could be of an immense economic importance for the people of the developing countries to resort to plant remedies. The aforementioned reports show that many plants, most of which are traditionally used for the treatment of different ailments in different parts of the world, are active against HIV replication at least *in vitro*. These reports are invaluable since they are the milestones for the discovery of new lead compounds and decision making at different levels. However, where many questions remain, it is premature to expect too much from the plethora of available leads at present. First of all it is important to note that a significant number of these studies are proof-of-concept experiments whose significance is not yet clear. Many of these studies have not also been pursued to the point that would enable pharmaceutical companies interested in this area to designate them as leads. Another fact in relation to this is that many findings are exclusively based on *ex vivo* biochemical or *in silico* assays. Such findings, if not confirmed further in cells and *in vivo* systems could potentially terminate in wasted effort. The cytotoxicity or selectivity profile of an antiviral compound or preparation should also be thoroughly evaluated for clinical efficacy and safety in HIV-infected patients, with confirmed HIV infection and a CD4 count <500 cells/µL. The polyherbal test preparation produced good symptomatic improvement within 6 months. The incidence and severity of symptoms such as diarrhoea, fatigue, anorexia, cough and fever decreased with drug treatment. There was a significant decrease in the mean viral load. The decrease in viral load was associated with an increase in mean CD4 count. With the exception of mild gastrointestinal adverse effects, the drug was well tolerated. Both patients and investigators rated the treatment as good or very good. It has been suggested that this herbal drug may have a good immunomodulatory effect and has potential as a co-therapeutic agent in the management of HIV infection (Usha et al., 2003).
Table 4. Anti-HIV medicinal plant extracts with unidentified constituents

<table>
<thead>
<tr>
<th>Source</th>
<th>Part of plant extract</th>
<th>Activity/Target</th>
<th>Potency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ailanthus altissima</em></td>
<td>Stem bark</td>
<td>HIV-1 fusion inhibition</td>
<td>74.9% at 100 µg/mL of extract</td>
<td>Chang and Woo, 2003</td>
</tr>
<tr>
<td><em>Atractylodes japonica</em></td>
<td>Root</td>
<td>HIV-1 fusion inhibition</td>
<td>72.8% at 100 µg/mL of extract</td>
<td>Min et al., 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 PR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.3% at 100 µg/mL of extract</td>
<td></td>
</tr>
<tr>
<td><em>Agrimonia pilosa</em></td>
<td>Whole plant</td>
<td>HIV-1 RT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 8.9 µg/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 PR</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 98.4 µg/mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>35% inhibition at 100 µg/mL of extract</td>
<td>Min et al., 2001</td>
<td></td>
</tr>
<tr>
<td><em>Cornus kousa</em></td>
<td>Stem and leaf</td>
<td>HIV-1 RT</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 8.3 µg/mL</td>
<td></td>
</tr>
<tr>
<td><em>Limonium tetragonum</em></td>
<td>Root</td>
<td>RNase H</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 7.6 µg/mL</td>
<td></td>
</tr>
<tr>
<td><em>Mallotus japonicus</em></td>
<td>Stem</td>
<td>HIV-1 RT</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 11.9 µg/mL</td>
<td></td>
</tr>
<tr>
<td><em>Clematis heracleifolia</em></td>
<td>Whole plant</td>
<td>HIV-1 PR</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 98.4 µg/mL</td>
<td></td>
</tr>
<tr>
<td><em>Syneilesis palmarata</em></td>
<td>Whole plant</td>
<td>HIV-1 PR</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 98.4 µg/mL</td>
<td></td>
</tr>
<tr>
<td><em>Crinum asiaticum var. japonicum</em></td>
<td>Root</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>ED&lt;sub&gt;50&lt;/sub&gt; = 12.5 µg/mL; SI = 16</td>
<td></td>
</tr>
<tr>
<td><em>Hyssopus officinalis</em></td>
<td>Leaves</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>Active at 50–100 µg/mL without appreciable cytotoxicity</td>
<td>Bedoya et al., 2002</td>
</tr>
<tr>
<td><em>Dictyophila viscosa</em></td>
<td>Aerial parts</td>
<td>Inhibited HIV-1 replication (estimated targets: early steps of virus replication, including virus-cell attachment, virus-cell fusion and cell-to-cell fusion)</td>
<td>Active at 10–400 µg/mL without cytotoxicity</td>
<td>Sanchez et al., 2002</td>
</tr>
<tr>
<td><em>Baccharis trinervis</em></td>
<td>Aqueous extract; part not specified</td>
<td>Inhibited HIV-1 replication (estimated targets: early steps of virus replication, including virus-cell attachment, virus-cell fusion and cell-to-cell fusion)</td>
<td>Active at 10–400 µg/mL without cytotoxicity</td>
<td>Sanchez et al., 2002</td>
</tr>
<tr>
<td><em>Combretum paniculatum</em></td>
<td>Leaves</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 5.2 µg/mL; SI = 6.4</td>
<td>Asres et al., 2001</td>
</tr>
<tr>
<td><em>Dodonaea angustifolia</em></td>
<td>Leaves</td>
<td>Inhibited HIV-2 induced cytopathogenicity</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 3.0 µg/mL; SI = 32</td>
<td></td>
</tr>
<tr>
<td><em>Ximenia americana</em></td>
<td>Stem bark</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 8.3 µg/mL; SI = 4.5</td>
<td></td>
</tr>
<tr>
<td><em>Bersama abyssinica</em></td>
<td>Root bark</td>
<td>Inhibited HIV-1 replication (estimated targets: early steps of virus replication, including virus-cell attachment, virus-cell fusion and cell-to-cell fusion)</td>
<td>Active at 10–400 µg/mL without cytotoxicity</td>
<td>Bedoya et al., 2002</td>
</tr>
<tr>
<td><em>Tubera lignosa</em></td>
<td>Aqueous extract (part not specified)</td>
<td>Inhibited HIV-1 replication (estimated targets: early steps of virus replication, including virus-cell attachment, virus-cell fusion and cell-to-cell fusion)</td>
<td>Active at 12.5–50 µg/mL without appreciable cytotoxicity</td>
<td>Bedoya et al., 2002</td>
</tr>
<tr>
<td><em>Sanguisorba minor magnolii</em></td>
<td></td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 15 µg/mL (After removal of polyphenolic compounds)</td>
<td>Au et al., 2001</td>
</tr>
<tr>
<td><em>Paeonia suffruticosa</em></td>
<td>Root</td>
<td>HIV-1 PR</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; = 45 µg/mL (After removal of polyphenolic compounds)</td>
<td>Lam et al., 2000</td>
</tr>
<tr>
<td><em>Prunella vulgaris</em></td>
<td>Flowers</td>
<td>HIV-1 PR</td>
<td>93.5% inhibition at 200 µg/mL of extract</td>
<td>Lam et al., 2000</td>
</tr>
<tr>
<td><em>Scutellaria baicalensis</em></td>
<td>Root</td>
<td>HIV-1 PR</td>
<td>91.1% inhibition at 200 µg/mL of extract</td>
<td>Lam et al., 2000</td>
</tr>
<tr>
<td><em>Hypstes lantanifolia</em></td>
<td>Aerial parts</td>
<td>HIV-1 RT</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 7 µg/mL</td>
<td>Matsuse et al., 1999</td>
</tr>
<tr>
<td><em>Tetrapetalus macrocarpa</em></td>
<td>Aerial part</td>
<td>HIV-1 RT</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 8 µg/mL</td>
<td></td>
</tr>
<tr>
<td><em>Combretum hartmannianum</em></td>
<td>Leaves</td>
<td>HIV-1 RT</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; = 66 µg/mL (non selective, equally towards p56&lt;sup&gt;a&lt;/sup&gt; tyrosine kinase)</td>
<td>Ali et al., 2002</td>
</tr>
<tr>
<td><em>Rubus rigidus</em></td>
<td>Root</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; ≥ 31.5 µg/mL; SI ≥ 2</td>
<td>Tshibangu et al., 2002</td>
</tr>
<tr>
<td><em>Acacia nilotica</em></td>
<td>Pods</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; ≥ 31.5 µg/mL; SI ≥ 2</td>
<td>Hussein et al., 1999</td>
</tr>
<tr>
<td><em>Batanales aegyptiaca</em></td>
<td>Bark</td>
<td>HIV-1 PR</td>
<td>Complete inhibition at 100 µg/mL</td>
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</tr>
<tr>
<td><em>Euphorbia granulata</em></td>
<td>Leaves</td>
<td>Inhibited HIV-1 induced cytopathogenicity</td>
<td>IC&lt;sub&gt;50&lt;/sub&gt; ≥ 62.50 µg/mL; SI ≥ 2</td>
<td></td>
</tr>
<tr>
<td><em>Maytenus senegalensis</em></td>
<td>Stem-bark</td>
<td>HIV-1 PR</td>
<td>48.5% inhibition at 100 µg/mL</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> HIV-1 protease; <sup>b</sup> HIV-1 reverse transcriptase; <sup>c</sup> HIV-1 integrase.
assessed before any further attention is given to it. Moreover, its compatibility and drug interaction of any sort with conventional antiretrovirals and other drugs commonly administered to AIDS patients should be studied as well.

Nevertheless it should be stressed that a number of natural products mainly derived from plants have proven effective in suppressing HIV replication and progress. Calanolide derivatives, pokeweed antiviral proteins and sulphated polysaccharides are only but a few of the compounds with excellent and promising antiviral activities. It is very much anticipated that anti-HIV cures and prophylactic preparations containing these natural products would soon be available.

The results and experiences with many of the anti-HIV natural products will inspire and motivate even more researchers to look for new leads from plants and natural sources. Many of the anti-HIV natural products have other medicinal values. These types of compounds may also be of interest as they can deal with both the virus and the various disorders that characterize HIV/AIDS.

REFERENCES


Charan RD, Munro MH, O’Keefe BR et al. 2000. Isolation and characterization of Myrianthus holstii lectin, a potent HIV-1


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